Citation:

Lichtenstein AH, Matthan NR, Jalbert SM, Resteghini NA, Schaefer EJ, Ausman LM. Novel soybean oils with different fatty acid profiles alter cardiovascular disease risk factors in moderately hyperlipidemic subjects. *Am J Clin Nutr.* 2006 Sep; 84(3): 497-504.

PubMed ID: 16960162

Study Design:

Randomized Controlled Trial

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

POSITIVE: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To assess the efficacy of novel soybean oils with modified fatty acid profiles, relative to soybean and partially hydrogenated soybean oils, on cardiovascular disease (CVD) risk factors in middle-agred and older moderately hypercholestrolemic and post-menopausal women and men.

Inclusion Criteria:

- More than 50 years of age
- LDL-cholesterol higher than 130mg per dL
- If female, post-menopausal.

Exclusion Criteria:

- Abnormal kidney, liver, thyroid and cardiac function
- Abnormal fasting glucose concentrations
- Taking medications know to affect blood lipid concentrations
- Smokers.

Description of Study Protocol:

Recruitment

30 subjects from the Boston area.

Design

- Randomized controlled trial, crossover design
- Subjects were provided with each of five experimental diets in random order for a 35-day period
- Subjects reported to the research facility three times per week for blood pressure (BP) and weight measurement
- Subjects ate one meal onsite and remaining food was supplied in containers
- Subjects were required to consume all food and were not allowed other food except water and non-caloric beverages
- Caloric requirements were estimated using the Harris-Benedict formula and were adjusted to maintain body weight
- Three times after day 28 of each diet phase, fasting blood samples were obtained for biochemical determinations, the mean value of the three-time points was reported and used for statistical purposes
- Diets were designed to provide 30% of energy from fat with two-thirds of the fat contributed by the

experimental oils.

Dietary Intake/Dietary Assessment Methodology

- Experimental oils were supplied by Solea Company (St. Louis, MO) and were soybean oil (SO), low-saturated fatty acid soybean oil (LoSFA-SO) developed by selective breeding, high oleic soybean oil (HiOleic-SO) developed by genetic modification and low α-linolenic acid soybean oil (LoALA-SO) developed by selective breeding
- Partially hydrogenated soybean oil (Hydrog-SO) was commercially available
- Chemical nutrient analysis of food provided for the diet was determined by Covance Laboratories America Inc (Madison, WI).

Nutrient Composition of the Experimental Diets as Determined by Chemical Analysis of Food[1]

Component	SO	LoSFA-So	HiOleic-So	LoALA-SO	Hydrog-So
Carbohydrate (% of energy)	52.1	53.5	54.6	52.1	53.9
Protein (% of energy)	16.7	17.0	17.7	16.8	17.2
Fat (% of energy)[2]	31.2	29.5	29.0	31.1	28.9
SFA (% of energy)[3]	6.52	4.91	5.76	6.75	7.25
14:0 (% of energy)	0.46	0.45	0.50	0.49	0.48
16:0 (% of energy)	3.48	2.10	2.78	3.58	3.48
18:0 (% of energy)	1.34	1.16	1.17	1.41	2.01
MUFA (% of energy)[3]	6.48	6.19	18.90	6.96	9.95
*c*16:1n-9 (% of energy)	0.15	0.10	0.13	0.14	0.12
*c*181n-9 (% of energy)	6.06	5.91	18.59	6.52	7.88
*t*181n-9 (% of energy)	0.18	0.09	0.08	0.23	1.85
PUFA (% of energy)[3]	12.74	14.60	2.82	13.51	8.66
*c*18:2n-6 (% of energy)	10.96	12.69	1.91	12.45	7.46
*t*18:2n-6 (% of energy)	0.25	0.21	0.14	0.16	0.47
*c*18:3n-3 (% of energy)	1.26	1.13	0.64	0.68	0.49
*t*18:3n-3 (% of energy)	0.18	0.34	0.11	0.13	0.20
Cholesterol (mg per 1,000kcal)	57	65	60	66	63
Fiber (g per 1,000kcal)	13.6	14.6	14.1	14.6	13.4

1 SO, soybean oil; LoSFA-SO, low-saturated fatty acid soybean oil; HiOleic-SO, high-oleic acid soybean oil; LoALA-SO, low-α-linolenic acid soybean oil; Hydrog-SO, hydrogenated soybean oil; SFA, saturated fatty acids; MUFA, monosaturated fatty acids; PUFA, polyunsaturated fatty acids, *c, cis; t, trans.*

2 Total dietary lipid-soluble components by Soxhlet extraction with the use of ether. The non-soponifiable fraction of the lipid-soluble components is represented by the difference between the total fat and the sum of fatty acids.

3 Fatty acids expressed as triacylglycerols (chloroform and methanol extraction and fatty acid methyl ester analysis by gas-liquid chromatography).

Blinding Used

Participants, laboratory personnel, and investigators were blinded to the order and identification of diet phases.

Intervention

Subjects were given five experimental diets in random order for 35-day diet phase:

- Soybean oil (SO),
- Low-saturated fatty acid soybean oil (LoSFA-SO)
- High oleic soybean oil (HiOleic-SO) d
- Low α-linolenic acid soybean oil (LoALA-SO)
- Partially hydrogenated soybean oil (Hydrog-SO).

Statistical Analysis

- An analysis of variance (PROC GLM) with main effect of diet and subject as repeated measure was carried out for each out come measure. Followed by Turkey's significant difference type of adjustment for the pairwise comparison among each of the five treatment regimens
- Effect of sex for each outcome variable was assessed by adding it as an additional main effect.

Data Collection Summary:

Timing of Measurements

Three times after day 28 of each diet phase, 12-hour fasting blood samples were collected.

Dependent Variables

- *Variable 1:* Plasma fatty acid profiles were extracted after addition of internal standard and analyzed using gas chromatograph and were expressed as molar percentage proportions of fatty acids relative to the internal standard
- *Variable 2:* Lipid and lipoprotein concentrations were measured with the use of Roche Diagnostics reagents and Wako Diagnostic reagents with a Hitachi 911 automated analyzer. VLDL was calculated as total cholesterol (LDL-cholesterol + HDL-cholesterol). Assays were standardized through the Lipid Standardization Program of the CDC (Atlanta, GA).

Independent Variables

Five diet manipulations with the following oils providing 20% of total fat intake (total fat intake was 30% of calories):

- Soybean oil (SO),
- Low-saturated fatty acid soybean oil (LoSFA-SO)
- High oleic soybean oil (HiOleic-SO)
- Low α-linolenic acid soybean oil (LoALA-SO)
- Partially hydrogenated soybean oil (Hydrog-SO) was commercially available.

Control Variables

- Body weight
- Blood pressure
- Gender.

Description of Actual Data Sample:

- *Initial N:* 30 (16 women and 14 men)
- *Attrition:* 12 of the original group recruited did not complete the study, but nine dropped out during phase one and were replaced with 10 other subjects
- *Mean age:* 63 years
- *Anthropometrics:* Mean BMI 26.2kg/m^2
- *Location:* Boston, Massachusetts area.

Summary of Results:

Phospholipid Fatty Acid Profiles at the End of Each Diet Phase[1]

Component	SO	LoSFA-SO	HiOleic-SO	LoALA-SO	Hydrog-SO
			mol %		
SFA					
Pooled	49.7±1.95[a](49.64)	49.16±2.26[a](49.25)	48.61±2.29[b](48.57)	49.28±1.89[a](49.23)	47.16±1.91[c](47.10)
Women	49.65±1.69 (49.64)	49.51±1.70 (49.51)	48.44±2.22 (48.60)	49.38±1.19 (49.38)	47.45±1.66 (47.20)
Men	49.27±2.26 (49.58)	48.72±2.84 (48.57)	48.81±2.45 (48.54)	49.16±1.95 (49.22)	46.83±2.19 (46.79)
MUFA					
Pooled	9.82±1.85[c,d] (9.45)	9.77±1.69[c] (9.82)	16.63±2.77[a] (16.30)	9.46±1.48[d] (9.26)	13.79±2.12[b] (13.73)
Women	9.48±0.79 (9.46)	9.5±1.08 (9.84)	16.23±1.87 (16.22)	9.18±0.82 (9.21)	13.60±1.73 (13.84)
Men	10.23±2.58 (9.22)	10.11±2.24 (9.78)	17.09±3.58 (17.23)	9.78±1.98 (9.29)	14.01±2.55 (3.73)
***c*18:1n-9**					
Pooled	6.19±0.86[c] (5.95)	6.11±0.85[c] (6.07)	12.79±2.64[a] (13.20)	6.08±0.75[c] (5.90)	6.59±0.91[b] (6.28)
Women	6.08±0.60 (6.04)	6.02±0.77 (6.07)	12.36±1.74 (12.71)	5.94±0.64 (5.69)	6.45±0.78 (6.09)
Men	6.33±1.09 (5.82)	6.21±0.96 (6.17)	13.28±3.42 (13.43)	6.26±0.85 (6.12)	6.75±1.04 (6.35)
***t*18:1n-9**					
Pooled	0.86±1.45[b,c] (0.54)	0.86±1.00[b] (0.58)	0.76±0.53[b] (0.71)	0.74±1.12[c] (0.52)	4.40±1.73[a] (4.49)
Women	0.61±0.32 (0.58)	0.67±0.34 (0.58)	0.87±0.60 (0.76)	0.56±0.30 (0.51)	4.34±1.45 (4.31)
Men	1.15±2.10 (0.45)	1.09±1.45 (0.57)	0.64±0.41(0.67)	0.96±1.62 (0.54)	4.48±2.07 (4.77)
PUFA					
Pooled	40.70±1.75[a] (40.94)	41.06±1.92[a] (41.58)	34.76±2.32[d](34.84)	41.26±1.80[a] (41.02)	39.04±1.26[b](39.43)
Women	40.88±1.44 (41.06)	40.98±1.89 (41.49)	35.33±1.85 (35.75)	41.44±1.89 (41.01)	38.95±1.07 (39.42)
Men	40.50±2.1 (40.82)	41.17±2.03 (41.65)	34.10±2.69 (33.75)	41.05±1.74 (41.26)	39.16±1.49 (39.49)
***c*18:2n-6**					
Pooled	23.77±2.82[b] (23.36)	23.97±3.08[b] (24.40)	17.35±2.08[d] (17.21)	24.56±3.09[a] (24.27)	22.27±2.27[c] (22.00)
Women	22.91±2.59 (23.15)	22.70±2.74 (23.97)	16.97±2.04 (17.27)	23.56±3.01 (23.73)	21.29±1.89 (21.32)
Men	24.77±2.83 (24.37)	25.54±2.83 (24.82)	17.78±2.21 (17.14)	25.7±2.86 (25.77)	23.4±2.20 (22.73)
***t*18:2n-6**					
Pooled	0.46±0.27[d] (0.37)	0.77±0.96[b] (0.49)	0.47±0.21[d] (0.41)	0.55±0.77[c] (0.28)	1.03±0.19[a] (1.04)
Women	0.44±0.21 (0.38)	0.95±1.26 (0.48)	0.52±0.26 (0.41)	0.63±0.93 (0.28)	0.99±0.16 (1.01)
Men	0.48±0.33 (0.37)	0.54±0.18 (0.52)	0.42±0.13 (0.42)	0.46±0.55 (0.26)	1.07±0.22 (1.10)
***c*18:3n-6**					

Pooled	0.07±0.03[a,b] (0.07)	0.07±0.04[a,b] (0.06)	0.07±0.03[a] (0.07)	0.08±0.06[a,b] (0.07)	0.06±0.03[b] (0.05)
Women	0.07±0.03 (0.06)	0.07±0.04 (0.06)	0.07±0.02 (0.07)	0.08±0.05 (0.07)	1.06±0.03 (0.05)
Men	0.07±0.03 (0.07)	0.06±0.04 (0.06)	0.08±0.03 (0.09)	0.08±0.07 (0.05)	0.06±0.03 (0.05)
***c*20:4n-6**					
Pooled	8.89±1.59[a] (8.70)	8.9±1.56[a] (9.20)	8.66±1.44[a,b] (8.84)	8.77±1.62[a,b] (8.66)	8.28±1.37[b] (8.19)
Women	9.65±1.14 (9.63)	9.56±1.22 (9.74)	9.18±1.18 (9.40)	9.56±1.37 (9.94)	8.92±1.24 (9.14)
Men	8.00±1.33 (7.97)	8.19±1.63 (8.47)	8.06±1.51 (8.30)	7.86±1.43 (7.59)	7.53±1.15 (7.31)
***c*18:3n-3**					
Pooled	0.25±0.06[b] (0.25)	0.25±0.04[b] (0.25)	0.29±0.08[a] (0.30)	0.20±0.06[c](0.19)	0.20±0.06[c] (0.19)
Women	0.25±0.05 (0.23)	0.24±0.05 (0.24)	0.29±0.06 (0.30)	0.19±0.04 (0.18)	0.20±0.05 (0.18)
Men	0.25±0.06 (0.26)	0.25±0.05 (0.26)	0.30±0.10 (0.30)	0.21±0.08 (0.19)	0.20±0.08 (0.20)
***c*20:5n-3**					
Pooled	0.56±0.18[b] (0.57)	0.55±0.19[b] (0.52)	0.81±0.27[a] (0.86)	0.43±0.17[c] (0.37)	0.48±0.21[c] (0.48)
Women	0.61±0.17 (0.63)	0.62±0.18 (0.58)	0.88±0.21 (0.88)	0.49±0.18 (0.50)	0.55±0.25 (0.53)
Men	0.49±0.17 (0.49)	0.47±0.18 (0.44)	0.73±0.32 (0.82)	0.35±0.13(0.31)	0.41± 0.13 (0.45)
***c*22:6n-3**					
Pooled	2.71±0.58 (2.74)	2.65±0.59 (2.50)	2.83±0.69 (2.86)	2.61±0.61 (2.57)	2.66±0.69 (2.56)
Women	2.96±0.61 (3.13)	2.86±0.63 (2.81)	3.10±0.67 (3.17)	2.80±0.61 (2.69)	2.83±0.76 (2.66)
Men	2.42±0.40 (2.48)	2.39±0.43 (2.42)	2.51±0.60 (2.42)	2.39±0.54 (2.27)	2.47±0.56 (2.46)
***trans* FA**					
Pooled	1.32±1.67[c,d] (0.90)	1.62±1.40[b] (1.08)	1.23±0.62[b,c] (1.09)	1.30±1.45[d] (0.79)	5.43±1.84[a] (5.58)
Women	1.05±0.38 (1.09)	1.62±1.26 (1.30)	1.39±0.70 (1.20)	1.19±1.03 (0.81)	5.33±1.53 (5.35)
Men	1.63±2.43 (0.83)	1.62±1.61 (0.99)	1.06±0.47 (0.94)	1.42±1.87 (0.75)	5.55±2.20 (5.86)

[1]All values are mean ±SD; medians in parentheses, SO, soybean oil; LoSFA-SO, low-saturated fatty acid soybean oil; HiOleic-So, high-oleic acid soybean oil; LoALA-SO, Low-α-linolenic acid soybean oil; Hydrog-SO, hydrogenated soybean oil; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; FA, fatty acids. *c, cis*; *t, trans*. All data were analyzed by ANOVA of rank values. ANOVA with main effects of diet and sex and subject as repeated measures indicated no significant effect of sex for the phospholipid fatty acid profiles. No sex-x-diet interaction terms were significant at $P<0.05$. Values in the same row with different superscript letters are significantly different, $P<0.05$.

Lipid and Lipoprotein Concentrations at the End of Each Experimental Diet Phase[1]

Component	SO	LoSFA-SO	HiOleic-SO	LoALA-SO	Hydrog-SO
Total cholesterol (mmol per L)					
Pooled	5.72±0.91[b]	5.59±0.96[b]	5.71±0.87[b]	5.74±0.81[b]	6.01±0.84[a]
Women[2]	6.02±0.86	5.85±0.90	6.02±0.95	6.07±0.80	6.38±0.80
Men	5.36±0.87	5.23±0.96	5.36±0.63	5.39±0.69	5.62±0.71
VLDL cholesterol (mmol per L)[3]					
Pooled	0.74±0.43	0.72±0.51	0.64±0.37	0.72±0.61	0.77±0.45

Women	0.73±0.28	0.69±0.40	0.68±0.38	0.67±0.32	0.77±0.35
Men	0.76±0.58	0.76±0.65	0.59±0.37	0.77±0.83	0.77±0.55
LDL-cholesterol (mmol per L)[3]					
Pooled	3.66±0.67[b]	3.53±0.77[b]	3.70±0.66[b]	3.71±0.64[a,b]	3.92±0.70[a]
Women	3.81±0.73	3.69±0.77	3.83±0.76	3.93±0.64	4.12±0.66
Men	3.47±0.54	3.33±0.75	3.56±0.51	3.47±0.55	3.7±0.70
HDL-cholesterol (mmol per L)[3]					
Pooled	1.32±0.32[a,b]	1.32±0.35[b]	1.36±0.33[a]	1.32±0.33[b]	1.32±0.32[a,b]
Women[2]	1.48±0.33	1.46±0.38	1.51±0.35	1.47±0.35	1.49±0.34
Men	1.13±1.18	1.14±0.21	1.20±0.19	1.15±0.21	0.15±0.19
HDL_2 cholesterol (mmol per L)					
Pooled	0.45±0.20	0.45±0.22	0.45±0.21	0.45±0.22	0.45±0.19
Women[2]	0.52±0.22	0.51±0.24	0.53±0.23	0.51±0.26	0.54±0.21
Men	0.36±0.13	0.36±0.14	0.36±0.15	0.39±0.16	0.36±0.12
HDL_3 cholesterol (mmol per L)[3]					
Pooled	0.87±0.15[b]	0.87±0.16[b]	0.91±0.14[a]	0.86±0.16[b]	0.87±0.15[b]
Women[2]	0.96±0.14	0.95±0.16	0.98±0.14	0.96±0.13	0.95±0.15
Men	0.77± 0.08	0.77±0.10	0.83±0.09	0.76±0.10	0.79±0.10
Triacylglycerol (mmol per L)[3]					
Pooled	1.67±0.81	1.65±0.92	1.16±0.74	1.73±1.01	1.73±0.79
Women	1.55±0.85	1.53±0.77	1.58±0.85	1.66±0.82	1.66±0.79
Men	1.81±0.76	1.82±1.11	1.65±0.61	1.80±1.21	1.79±0.82
ApoB (mmol per L)					
Pooled	98±16[b]	95±17[b]	97±16[b]	99±14[a,b]	103±14[a]
Women	100±17	98±16	101±18	102±16	106±15
Men	96±16	91±18	93±13	95±11	99±12
ApoA-I (mmol per L)[4]					
Pooled	121±17[b]	119±17[b]	124±16[a]	120±17[b]	121±15[b]
Women[2]	131±16	128±15	133±15	129±16	130±15
Men	108±10	107±9	113±9	109±9	111±8
Lp(a) (mmol per L)[3]					
Pooled	14±15	14±14	15±15	15±16	15±15
Women	20±18	20±16	21±18	21±18	21±18
Men	7±6	7±6	8±7	8±8	8±8
T:HDL-C					
Pooled	4.55±1.00[b]	4.43±1.09[b]	4.37±0.98[b]	4.55±0.99[a,b]	4.72±0.93[a]
Women	4.24±1.03	4.20±10.5	4.16±1.06	4.32±1.04	4.46±1.00
Men	4.81±0.89	4.73±1.12	4.55±0.75	4.81±0.89	4.99±0.81
LDL-C:ApoB					

Pooled	1.45±0.15	1.44±0.16	1.47±0.13	1.45±0.18	1.46±0.17
Women	1.48±0.13	1.46±0.15	1.47±0.14	1.49±0.14	1.50±0.13
Men	1.14±0.17	1.41±0.18	1.47±0.13	1.41±0.21	1.42±0.20
HDL-C:ApoA-1					
Pooled	0.42±0.06	0.43±0.07	0.42±0.06	0.42±0.06	0.42±0.06
Women	0.43±0.06	0.44±0.07	0.44±0.07	0.44±0.07	0.44±0.06
Men	0.40±0.05	0.41±0.07	0.41±0.05	0.41±0.06	0.40±0.05
CRP (mmol per L)[3]					
Pooled	3.70±4.60	4.12±5.59	4.63±7.07	3.73±4.94	4.50±6.89
Women	4.35±5.37	5.12±7.31	4.88±6.40	4.88±6.40	5.00±6.59
Men	2.95±3.56	2.87±1.62	4.37±8.26	2.41±1.86	3.93±7.44

[1]All values are mean ±SD; SO, soybean oil; LoSFA-SO, low-saturated fatty acid soybean oil; HiOleic-So, high-oleic acid soybean oil; LoALA-SO, Low-α-linolenic acid soybean oil; Hydrog-SO, hydrogenated soybean oil; Apo, apolipoprotein; CRP, C-reactive protein; Lp(a), lipoprotein(a). Values in the same row with different superscript letters are significantly different, $P<0.05$.

[2]ANOVA with main effects of sex and diet and subject as repeated measures showed a significant effect of sex ($P<0.05$) for these variables: No sex-x-diet interaction terms were significant at $P<0.05$

[3]Appropriate transformations of the data (log VLDL, LDL, HDL_3, triacylglycerol and CRP; inverse HDL; square root Lp(a); and ranks of ApoA-1) were made before ANOVA with the main effect of diet and subject as repeated measure followed by Tukey's T-test for multiple comparisons.

[4]Data were analyzed by using non-parametric methods (signed-rank test).

Other Findings

- The phospholipid fraction of plasma at the end of each phase reflected the predominate fat in the diet and was evidence of dietary compliance
- LDL concentrations were highest after subjects consumed the Hydrog-SO diet and LoALA-SO than after the other diets
- Patterns were the same for men and women
- After subjects consumed the HiOleic-SO, *cis* monounsaturated fatty acids represented approximately 17% of the total fatty acids, this was 40% higher than after subjects consumed SO, LoSFA-SO and LoALA-SO
- Plasma phospholipid trans fatty acids were approximately 3.5 times higher when subjects consumed Hydrog-SO enriched diet relative to the other diets
- The pattern of response to the diet was reflected in plasma ApoB concentrations. In contrast, HDL concentrations were NS different between the diets of modified fats and either the SO or Hydrog-SO diet, although for men, HDL concentrations of the HiOleic-SO diet were significantly greater than with the SO diet.
- Ratios of LDL to ApoB and HDL to ApoA-I were similar at the end of diet phases
- NS effect was observed on VLDL, triacylglycerol, Lp(a) or CRP.

Author Conclusion:

All varieties of soybean oils resulted in more favorable lipoprotein profiles than did the partially hydrogentated form. These soybean oils may provide a viable option for reformulation of products to reduce the content oftrans fatty acids.

Reviewer Comments:

None.

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions		
1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	Yes
4.	**Was method of handling withdrawals described?**	Yes

4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	Yes
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	Yes
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	Yes
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	Yes
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes

7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes